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Several genetic alterations have been identified in breast cancer, yet the molecular mechanisms leading to breast tumorigenesis are unknown. In order to develop a mouse model system that can dissect the genetic pathways involved in breast tumor development, we are adapting a somatic gene transfer system based on avian leukosis virus A (ALV-A) expression vectors and the TVA receptor for ALV-A. Transgenic mice have been generated that target tv-a to the mammary epithelial cells using MMTV and WAP promoters. Mammary epithelial cells from these transgenic mice are susceptible to infection both in vivo and in vitro. The animals developed should be valuable for gene delivery using the ALV-TVA vector system.

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## INTRODUCTION

A large body of genetic lesions, both somatic and hereditary, have been identified in human breast cancer. These include *p53*, *BRCA1,* and *BRCA2*, *PTEN/MMAC1*, *neu/ErbB2/HER2*, *ErbB1/EGFR*, *PRAD-1/cyclin D1*, *Mdm2*, and *c-myc*. The molecular events responsible for specific steps in the initiation and progression of breast cancer are, at present, unclear, but it is generally thought that breast cancer evolves from ductal hyperplasia, hyperplasia with atypia, carcinoma in situ, invasive carcinoma, and, finally, to metastatic disease. Many animal models have been created to investigate the importance of genetic lesions in tumor evolution since transgenic and knockout techniques can be used to create mice that are identical in genetic background except for one or a few genes that are to be examined. My research is designed to use a viral gene transfer system (Federspiel et al., 1994) to overcome some of the limitations that are currently faced by germline manipulations. This system is based on the fact that avian leucosis virus subgroup A (ALV-A) can transduce mammalian cells without production of viral particles when the gene encoding the avian receptor, TVA, is ecotopically expressed in these cells (reviewed by Fisher et al., 1999). Consequently, after generating a single transgenic line expressing the *tv-a* in a target tissue, individual and combinatorial effects of any oncogenic genes can be examined using ALV-A as a delivery vehicle. This system has been demonstrated to work in modeling gliomas (Holland et al., 2000; Holland et al., 1998; Holland & Varmus, 1998). I am adapting this approach to the mammary gland to study the effects of several breast cancer genes, including *Brca-1*, *p53*, and *HER2/neu*, in mammary oncogenesis.

## BODY

### Task 1. Establish mammary-specific gene transfer methods (months 1-6).

Using a polyclonal antibody against TVA in immunohistochemistry, I have selected, among over twenty founder lines, four transgenic lines that express *tv-a* from mammary targeted promoters (MMTV and WAP). Cultured mammary epithelial cells prepared from one MMTV-*tv-a* line (MA) can be infected with RCAS (a derivative of ALV-A) encoding alkaline phosphatase (RCAS-*AP*). The percentages of infected cells can be significantly increased (from 5-10% to 30%) by the use of concentrated viruses and by multiple infections. Preliminary results show that these cells can be doubly infected, as we anticipated. Direct injection of RCAS-*AP* into mammary glands in mid-pregnant transgenic mice resulted in AP staining in some ducts, suggesting that these cells are susceptible to infection in vivo.

### Task 2. Introduce protooncogenes/oncogenes and dominant-negative TSGs into mammary glands in an effort to produce tumors (months 3-24)

I have made several RCAS viruses to express oncogenes and dominant negative tumor suppressor genes that are implicated in breast cancer. Among the viruses generated are RCAS expressing a mutant form of *HER2/neu*, *c-myc*, *Akt*, *cyclin D*1, a dominant-negative mutant of *p53*, *E-cadherin*, and *TGF-beta receptor 1*. Infection of two MMTV-*tv-a* mice with RCAS viruses encoding Wnt-1, cyclin D1, and FGF-3 led to hyperplasia and dysplasia in seven months, while two non-transgenic control mice did not display any ductal lesions. It remains to be determined what virally expressed genes are present in these lesions.

### Task 3. Generate a mouse line carrying a floxed *Brca2* allele in collaboration with Anthony Wynshaw-Boris (months 1-12).

A targeting construct was made with both negative and positive selection markers. Transformation of this construct into bacteria expressing the gene encoding the Cre recombinase was found to yield a smaller plasmid, with the size expected for the correct excision of the intervening DNA flanked by the loxP sites. Transfection of this construct into ES cells, followed by positive and negative selections, yielded 210 stable colonies. However, assays using Southern hybridization and PCR screening did not detect any colonies that had undergone homologous recombination. Since several other laboratories have already generated targeted mice carrying floxed *Brca-2*, I have discontinued further efforts to generate this line of animals.

**Task 4. Delete floxed TSG in mammary glands at targeted times in hopes of generating tumors (months 12-24).**

I have obtained a Cre reporter line—ZAP (Lobe et al., 1999), which expresses *LacZ* or *AP* depending upon the absence or presence of Cre, and have crossed it into line MA. Mammary epithelial cells prepared from this line will be infected with RCAS-*Cre* to determine the efficiency of deletion of floxed *LacZ*.

I have imported mice carrying floxed *Brca-1* (Xu et al., 1999) and have bred the floxed allele into the MA line. The resulting *tv-a* TG, $Brca^{fl/fl}$ will be used for infection with RCAS-*Cre* in combination with oncongenic viruses such as RCAS-DN *p53*, RCAS-*neu* and RCAS-*c-myc*.

**Task 5. Express protooncogenes/oncogenes and inactivate floxed TSGs in the mammary glands in order to generate tumors (months 24-36).**

This section represents future work.

**Task 6. Characterize tumors generated in the course of this study (months 24-36).**

This section represents future work.

## KEY RESEARCH ACCOMPLISHMENTS

1. I have created transgenic lines expressing *tv-a* from the MMTV promoter
2. I have created transgenic lines expressing *tv-a* from the WAP promoter
3. I have shown that mammary epithelial cells from MMTV-*tv-a* mice can been infected by avian leucosis viruses.
4. I have created ALV viruses expressing genes encoding HER2/neu, myc, Akt, cyclin D1, and polyoma middle T antigen.

## REPORTABLE OUTCOMES

1. *tv-a* transgenic lines that express TVA in the mammary epithelial cells.
2. ALV viruses expressing genes HER2/neu, myc, Akt, cyclin D1, and polyoma middle T antigen.

## CONCLUSIONS

I have generated transgenic mice expressing *tv-a* from mammary-specific promoters. The mammary epithelial cells from these mice can be infected with RCAS viral vectors. Experiments are underway to determine more efficient methods to infect mammary cells. After the system is better characterized, it should allow more rapid evaluation of the impact of single and combinatorial genetic lesions that have been implicated in breast cancer.

**A new direction of this research**---Metastasis is what ultimately kills breast cancer patients, but the molecular cues for metastasis are unknown. Since MMTV-*Wnt-1* mice develop mammary tumors that usually do not metastasize, I would like to try to induce metastasis of these tumors. MMTV-*tv-a* has been bred into MMTV-*Wnt-1* mice. A number of candidate genes (such as genes encoding polyoma middle T, HER2/neu, members of the TGF beta pathway, and dominant-negative E-cadherin) that are known to play roles in tumor metastasis will be tested first by injecting viruses carrying these genes directly into the developing tumors. If successful, additional candidates will be tested. This model system, once developed, would be an ideal approach to dissect pathways involved in tumor invasion and metastasis and could also be useful for animal testing of mechanism-based therapeutic drugs in both treatment and prevention.

**Other achievements not proposed in the approved proposal**-- *PTEN* encodes a phosphatase that removes the D3 phosphate of phosphatidylinositol (3, 4, 5)-triphosphate, reverting the effect of PI3K and, therefore, inhibiting AKT, a serine/threonine kinase that inhibits apoptosis. *Pten* is mutated in many human cancer types, including breast cancer. In mice, inactivation of *Pten* predisposes to multiple neoplasias, but high-grade mammary adenocarcinomas are rarely observed (Di Cristofano et al., 1998; Podsypanina et al., 1999; Stambolic et al., 2000; Suzuki et al., 1998). I have generated a mouse model in which the role of *Pten* can be studied in breast cancer by crossing *Pten* heterozygous mice to MMTV-*Wnt-1* transgenic mice that routinely develop mammary carcinomas. Female *Wnt-1* transgenics that were heterozygous for *Pten* developed mammary tumors at an accelerated rate compared to those with the wild type *Pten* alleles. In males, the frequency of tumors was markedly increased in the *Wnt-1* transgenics heterozygous for *Pten*. In most tumors arising in Pten heterozygotes, the Pten wild-type allele was lost, suggesting that cells that lack *Pten* function have a growth advantage over cells retaining a wild type allele. Loss of *Pten* is accompanied by increased levels of phosphorylated AKT. This animal model may be useful in testing radiation and chemical therapies against tumors carrying a *Pten* mutant.

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